evidence that thio-phoshate is indeed incorporated into the DNA and RNA of a variety of organisms such as bacteria, yeast, and goldfish in the experimental sections of the parent application and the present patent application. Claim 9 is no different in implying that thio-phosphate is incorporated into nucleotide precursor pools that subsequently modify nucleic acids in vivo.

The emphasis in Claim 9 is its use to modify the nucleic acids of complex organisms. No specific function is addressed other than the unique ability to chemically alter and/or tag these nucleic acids in vivo. Rather in claim 9 attention is drawn to different approaches for substituting phosphate in the organism with thio-phosphate. There are two basic approaches: 1) replacing the orthophosphate in the organism's feed with thio-phosphate OR 2) adding an excess of thio-phosphate to the organism's system so that it can compete with the ortho-phosphate present for uptake. When considering replacing the normal feed source with thio-phosphate feed one can potentially add it to a solid feed source or to a liquid feed source. In particular, thio-phosphate could be added to a low phosphate feed source such as a synthetic feed or a feed source depleted in phosphate. This is analogous to preparing bacterial media that are synthetic and/or depleted in phosphate as described in the parent application and the present application. Obvious synthetic sources for complex organisms would be those analogous to protein powders, starches, and liquid meal replacements in common use today.

The parent application which is incorporated by reference describes the uptake of thio-phosphate into goldfish intestinal DNA. A large excess of thio-phosphate in the fish water was needed to compete with internal sources of phosphate. Excess here means relative to the amount present in normal fish feed which is added to the aquarium or about a 250 fold excess. Intestinal cells turnover rapidly making it possible to test for thio-phosphate incorporation into the DNA of these cells. Thus the method has actually been reduced to practice contrary to the examiner's claims. While incorporation into RNA was not directly demonstrated it can be inferred. It is presumably easier to incorporate into RNA as no cellular turnover is required for thio-phosphate incorporation. Isolation of modified DNA from complex organisms however, is easier to demonstrate. These studies do however demonstrate that it is more difficult to replace thio-phosphate for phosphate in complex organisms owing to competition with internal sources.

Claim 10

Claim 10 deals with the use of thio-phosphate to enhance the stability of RNA in vivo by creating nuclease resistance phosphorothioate linkages in the RNA and thereby leading to enhanced protein synthesis. Stabilization of mRNA can increase the accumulation of mRNA in the cell which is often directly proportional to protein synthesis levels. In the present invention, all mRNAs synthesized by RNA polymerase in the cell are modified by nuclease resistant linkages. Note this is in contrast to the examiner's assertion that a single mRNA is modified. This is a more global enhancement of mRNA levels then what you might expect by say increasing the promoter output of a specific gene. In the end though not all mRNAs are equally enhanced in terms of their respective protein levels.

What was noted in the experimental section of the present application, is that the optimal amount of thio-phosphate to enhance protein expression is less than 100% substitution for normal phosphate. This is because high ratios of thio-phosphate to unmodified phosphate result in a decrease in total protein synthesis. Some of this may be attributed to a slight reduction in growth. Surprisingly enough even though total protein synthesis is decreased some mRNAs can still be enhanced at higher ratios of thio-phosphate to phosphate (table and paragraph 2 of example II). This suggests that competition is occurring between mRNA species for translation possibly due to limiting protein factors. Competition also occurs at lower concentrations of thio-phosphate to phosphate where total soluble protein synthesis is not significantly increased even though some proteins can be increased as much as ten fold. In addition to protein translation factors that may be limiting there may be limitations in the amount of total protein a cell can produce in its interior. In particular, at high concentrations of protein, inclusion bodies occur readily in bacteria (paragraph 5 of example II). This is less of an issue for secreted proteins which have no space barrier and for eukaryotic cells which are larger.

The method preferentially enhances the expression of those mRNAs that are turned over more frequently. This is because these mRNAs have the opportunity to incorporate the modified phosphate more readily and as such they reach steady state more quickly. They also stand to benefit the most. Let's say an mRNA has a half-life of 5 minutes but now because of chemical modification it is increased to 10 minutes. A longer lived mRNA with a half-life of 1 hr may only be enhanced to say

1.5 hrs or even less. This means that there are now more of the "unstable" mRNAs relative to the stable mRNAs inside the cell. These "unstable"mRNAs can now compete more effectively for translation factors resulting in the preferential enhancement of their respective protein levels. So in practical terms the method is useful for enhancing the protein expression levels for genes with mRNAs that have short to average half-lives compared to those with longer half-lives in the cell. This is supported by the experimental data showing that the bacterial AP gene is preferentially enhanced relative to the beta-galactosidase gene (table, experimental section 2 paragraphs 5 &6). This is also supported by the results in yeast showing that heterologous expression of the bacterial AP gene compared yeast native extracellularhydrolases such as lipase and presumably amylase as well.

Claim 8

While claim 8 has been accepted I have modified it further to be sure that tissue culture cells are not excluded. While these are not micro-organisms per se they are sometimes lumped together with them because of similar design in culturing, i.e. clones of cells. Thio-phosphate can be anticipated to be incorporated into virtually all cell types based on its universal mechanism of action. The parent application and present application via incorporation of the parent by reference demonstrate that goldfish are viable for at least two days with thio-phosphate as the only source of phosphate in the media. Whole organisms are generally more sensitive to toxicity than tissue cultures cells giving a good indication of the generally applicability of the method and potential viability of cells.

The claims have been modified below to more succinctly embody the invention. These claims will hopefully meet the examiner's requirements.

I wish to declare and make a verified statement that the modified claims include no new matter as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825 (d).

Respectfully submitted,

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CLAIMS AMENDMENTS

What is claimed is:

Claims 1-7 (canceled)

8. (currently amended)

A method for stabilizing and/or accumulating cellular RNA in vivo resulting in an increase in the total amount of cellular mRNA per cell as well as the protection of RNA from degradation during subsequent isolation procedures comprising:

1) preparing microbial or cell culture media depleted of phosphate
2) adding thiophosphate as the sole source of phosphate to the media
3) culturing micro-organisms or cells in the modified media for a sufficient time such that most mRNAs and/or rRNAs are modified with phosphorothioate linkages

9. (currently amended)

A method for chemically modifying the RNA/DNA inside the cells of complex organisms comprising:

~~1) identifying the source of organic phosphate in the organism's diet~~

~~2)~~ 1) substituting the normal source of phosphate with thio-phosphate by replacing it with thio-phosphate and/or ~~removing the organic phosphate source from the diet by substituting thio-phosphate for organic phosphate in the diet alternatively~~ adding an excess of thio-phosphate ~~to the diet~~ such that thiophosphate ~~it~~ effectively competes with the orthophosphate ~~normal phosphate~~ present for incorporation into cellular nucleotide pools

~~3)~~ 2) introducing thio-phosphate into the organism ~~feeding thio-phosphate to the organism~~ by adding it to solid feed, ~~or by~~ feeding the organism a liquid source of thio-phosphate, or ~~alternatively by~~ injecting thiophosphate directly into the organism.

10. (currently amended)

A method to enhance the in vivo ~~synthesis of a~~ protein expression ~~or a group~~ of mRNAs with short to average half-lives relative to those with longer half-lives ~~proteins by stabilizing their corresponding mRNA(s) in vivo~~ comprising:

1) growing microbial cells in thio-phosphate containing culture media
2) determining the optimal ratio of thio-phosphate to inorganic phosphate in the culture media that enables ~~such that~~ the desired mRNAs ~~mRNA(s)~~ ~~is~~ to preferentially compete for translation. ~~stabilized and translated inside cells~~
3) determining the optimal time point to induce stabilization of the desired mRNAs ~~mRNA(s)~~ via the addition of thio-phosphate containing media to ~~growing~~ cultures that results in maximal protein synthesis of the corresponding gene products ~~product(s).~~

11. (currently amended)

The method of claim 10 wherein the cells used to generate the proteins ~~or protein of interest~~ are prokaryotic or eukaryotic in origin.

12. (currently amended)

The method of claim 10 wherein the proteins include a ~~or~~ protein ~~of interest is~~ produced using a recombinant DNA or viral expression vector or ~~is~~ a viral protein produced by an intact virus.

13. (amended)

The method of claim 10 wherein the enhancement of one or more proteins in cells results in an increase in a rate limiting enzyme(s) needed for the synthesis of a given organic chemical fermentation product.

14. (cancelled)